MicroRNA-146a polymorphism is not associated with cardiovascular disease in the elderly

Polimorfismo MicroRNA 146a não está associado a doenças cardiovasculares em idosos

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Abstract

Introduction: Cardiovascular diseases (CVD) figure among the most significant causes of morbidity and mortality in the world and, among genetic factors, the literature has demonstrated the crucial role of miRNAs and the relationship of physical activity with this pathology. **Objective:** To investigate the relationship between the functional capacity of exercise, the level of physical activity, and the polymorphism in the miRNA-146a gene in elderly individuals with and without CVD. **Methods:** This study, developed in a city in the southern region of Brazil, is characterized as cross-sectional. The sample for this study comprised 342 participants, aged 60 or over. The following aspects were analyzed: anthropometric characteristics, genetic profiles, diagnosis of CVD, functional capacity, and the level of physical activity. **Results:** A statistically significant association was observed between CVD and body mass index (BMI) ($\aleph^2$ = 14.278; p = 0.0003), and 40.6% of elderly individuals with CVD were obese, while 31.5% of the normally developed elderly participants presented normal BMI. However, the genotype frequencies (p = 0.546; $\aleph^2$ = 1.211) and 6MWT (p = 0.311; $\aleph^2$ = 1.025) did not show a statistically significant association with CVD. **Conclusion:** Our results suggest that the polymorphism in the miRNA-146A (rs2910164) and functional capacity are not associated with CVD in the elderly. However, the BMI did demonstrate an association with this disease.

Keywords: Cardiovascular disease. Elderly. Genetic polymorphism. MicroRNA. Physical activity.

Resumo

Introdução: *As doenças cardiovasculares (DCV) figuram entre as causas mais significativas de morbimortalidade no mundo e, dentre os fatores genéticos, a literatura tem demonstrado o papel crucial dos miRNAs e a relação da atividade física com essa patologia.* ***Objetivo:*** *Investigar a relação entre a capacidade funcional do exercício, o nível de atividade física e o polimorfismo no gene miRNA-146a em idosos com e sem DCV.* ***Métodos:*** *Este estudo, desenvolvido em um município da região sul do Brasil, caracteriza-se como transversal. A amostra deste estudo foi composta por 342 participantes, com idade igual ou superior a 60 anos. Foram analisados os seguintes aspectos: características antropométricas, perfis genéticos, diagnóstico de DCV, capacidade funcional e nível de atividade física.* ***Resultados:*** *Observou-se associação estatisticamente significativa entre DCV e índice de massa corporal (IMC) (κ^2 = 14,278; p = 0,0003), sendo que 40,6% dos idosos com DCV eram obesos, enquanto 31,5% dos idosos normalmente desenvolvidos apresentaram IMC normal. No entanto, as frequências genotípicas (p = 0,546; κ^2 = 1,211) e TC6 (p = 0,311; κ^2 = 1,025) não mostraram associação estatisticamente significante com DCV.* ***Conclusão****: Os resultados do presente estudo sugerem que o polimorfismo no miRNA-146A (rs2910164) e a capacidade funcional não estão associados à DCV em idosos; no entanto, o IMC demonstrou associação com essa doença.*

Palavras-chave: *Doença cardiovascular. Idoso. Polimorfismo genético. MicroRNA. Atividade física.*

Introduction

Physical therapy plays an important role in cardiovascular rehabilitation and in the prevention of disease in the aging process. Cardiovascular diseases (CVD) are among the most significant causes of morbidity and mortality in the world. The World Health Organization (WHO) reports that, in 2011, approximately 17 million people died as a result of these problems.[1,2] Sullivan et al.[3] and Mendes et al.[4] identified different, modifiable and non-modifiable aspects in the prognosis of the disease, such as age, gender, smoking habit, body mass index (BMI), lifestyle and genetic factors.[1-5]

As aging is considered a multifactorial process, with morphological, functional and biochemical changes in the body as a whole, it is possible that the elderly individual could develop a reduced capacity to adapt to the environment, generating greater fragility.[5] The act of walking is one of the most common daily tasks and the main one for individuals to remain physically independent; thus, the walk test is seen as an important step for the measurement of functional capacity in the elderly.[6]

In this aging process, an increment in body fat occurs and, consequently, morbidities may occur due to a variety of metabolic changes, a fact well established in the literature, that may influence the functional capacity of the individual.[7]

There are still a number of factors that have been established with a strong relationship to the manifestation of CVD, such as genetic, environmental and lifestyle factors. These risk factors include smoking, high blood pressure, high levels of cholesterol and/or reduced levels of high-density lipoproteins (HDL), diabetes mellitus, excess weight/obesity, stress, family history and genetic racial factors, gender, as well as a sedentary lifestyle.[8,9]

Moreover, functional capacity may be cited as a fundamental factor for the elderly to be able to live independently, being able to perform physical and mental activities to preserve their basic, essential activities.[10] In addition to the practice of physical activity, physically active individuals exhibit lower rates of morbidity from chronic diseases than sedentary individuals, with a relationship between the level and practice of physical activity and better quality of health.[11]

Thus, the six-minute walk test (6MWT) is a widely used instrument for combining operational ease and functional representation in the assessment of the functional exercise capacity of the physically active, sedentary individuals and those with chronic diseases. Hence the assessment of the level of daily physical activity using the Modified Baecke Questionnaire of Habitual Physical Activity for the Elderly (mBQ), due to its easy reproducibility and low cost.[12,13]

Of the genetic factors, several biomarkers have been proposed. In this regard, different levels of expression of miRNAs were identified in various body tissues, and their involvement in the pathogenesis of disease.[14]

Micro RNAs (miRNA) correspond to small sequences of 21-23 nucleotides that have repressive activity at the post-transcriptional level, because they associate

the complementary sequences of target messenger RNA, leading to their degradation. In addition, the expression of mRNA can be redirected by the presence of polymorphisms (SNPs). Single nucleotide polymorphisms (SNPs) are the most common types of variation in the human genome and, although there is often no direct relationship between the manifestation of disease and SNPs, research has identified several polymorphisms involving the molecular bases of genetic diseases.[15]

The miRNA-146A was associated with the progression and prognosis of diseases such as cancer and autoimmune disorders.[16] However, studies involving this marker in CVD are scarce. The gene that encodes the miRNA-146a is located on chromosome 5q33, and its expression exerts an inflammatory modulation effect by acting on the NF-κB and IRAK-1 pathways, associated with the tumor necrosis factor; thus, it is not unreasonable to suggest that it might be related to the development of CVD.[2,17]

In fact, Xu et al.18 demonstrated that SNP rs2910164 of the miRNA-146a gene seems to modify its expression, with the change from G to C resulting in less formation of miRNA-146a, thus contributing to a failure in the regulation of the inflammatory process and leading to susceptibility to CVD.[18]

Thus, the investigation of miRNA-146a as a biomarker for CVD would be of great value to the Brazilian population, as well as the relationship between genetic factors and modifiable aspects, such as an individual's sedentary lifestyle, habits and overall lifestyle. Thus, the objective of the present study was to investigate the relationship between the functional capacity of exercise, the level of physical activity, and the polymorphism in the miRNA-146a gene in elderly individuals with or without CVD.

Methods

Design and study participants

A cross-sectional study in respect of aging and longevity was performed between September 2009 and January 2013, developed in a city in the southern region of Brazil, with the aim of assessing the sociodemographic factors and health indicators in this local population. This project consisted of a total sample of 506 elderly subjects, which is representative of the total local population of 43,610 elderly individuals, and the convenience sample for this study was composed of the elderly people in this project. Individuals registered in 38 Basic Health Units (UBS) in the urban area of Londrina, where the calculated study sample was 342 individuals, considering a confidence interval of 95% and sampling error of 5%. The elderly subjects were selected from the records of the basic health units in a stratified, random manner. Consequently, 454 individuals were included.

It comprised a population of individuals with an independent lifestyle, of both genders, aged 60 or over, who are classified as levels 3 and 4 in the Functional Status proposed by Spirduso,[19] all of them included in the project.

The participants voluntarily accepted the invitation to participate in the study and signed a consent form which was approved, together with this research, by the Human Research Ethics Committee (HREC).

The criteria for inclusion were: aged 60 years and over, complete independence in daily activities; absence of severe comorbidities that would prevent completion of the test, including pre-diagnosed incapacitating cardiac and orthopedic diseases. Individuals with neuromusculoskeletal disease and mental limitations that could impair the understanding and performance of the tests involved in the study, were excluded.

Anthropometric data

Weight and height were measured using anthropometric scales manufactured by Filizola® (Filizola, São Paulo, Brazil). The body mass index (BMI) was obtained through the calculation of weight/height² according to the protocol established by Guedes and Guedes.[20] For the measurement of the height of each participant, a standard positioning was observed, with erect, postural alignment, limbs parallel to the body and feet together; the heels, buttocks, scapular waist and occipital region should be in contact with the measuring scale. Lastly, to obtain the measurement, the individual should inhale as far as possible.

To obtain their weights, the participants in this study were then positioned in standing, at the central point of the scale, with the feet positioned in line with the width of the hips and body weight evenly distributed; in front of the participant, a fixed point helped to avoid oscillations in measurement.

Diagnosis of heart disease

The diagnosis of heart disease was performed via the cardiological medical report, as well as through an analysis of the drugs that the participants reported using. Among the drugs used, a detailed analysis was performed in which individuals were subdivided into hypertension, dyslipidemia, hypertension, and dyslipidemia and lastly, individuals without vascular cardiopathy.

Lipid profile

The levels of total cholesterol, HDL, LDL and triglycerides in the plasma collected from the elderly participants in the study were analyzed. For the values of the lipid profiles of the elderly, those advocated by the Brazilian Society of Cardiology, the Brazilian Society of Clinical Analysis, Brazilian Society of Clinical Pathology/Laboratory Medicine and the Brazilian Society of Biomedicine - V Brazilian Guidelines on Dyslipidemia and Prevention of Atherosclerosis, were employed.[21]

Diagnosis of Diabetes Mellitus type 2

The diagnosis of diabetes mellitus type 2 (DM2) was made from the reports provided by the participants themselves, from an analysis of medical records and by means of blood tests (fasting glycemia and glycated hemoglobin).

The data obtained were analyzed in accordance with the values established by the Brazilian Society of Diabetes,[22] and the elderly subjects were subdivided into three groups: non-diabetic (glycemia ≤ 99 mg/dL and glycated hemoglobin < 6.5%), pre-diabetic (fasting blood glucose between 100 mg/dL and 125 mg/dL) and lastly, diabetic (fasting glucose ≥126 mg/dL and glycated hemoglobin > 6.5%).

Diagnosis of hypertension

The individuals participating in the project reported in advance whether or not they were hypertensive, and if they made use of anti-hypertensive medication. A confirmation of systemic arterial hypertension (SAH), as established by the WHO/ISH in 1999, was performed: systolic arterial pressure = 140 mmHg and diastolic arterial pressure ≥90 mmHg. For the normotensive individuals, the measurement of pressure was <140 mmHg for systolic blood pressure and <90 mmHg for diastolic blood pressure.[23] For the purposes of uniformity, blood pressure values were obtained with the elderly seated at rest for 5 minutes, and arterial blood pressure was measured in the right arm.

Assessment of the level of daily physical activity

For the assessment of the level of daily physical activity of the elderly participants in the project, we used the Modified Baecke Questionnaire for the Elderly (mBQ), which evaluates the data from three specific areas, namely activity performed at home, sports activities and leisure activities.[24] Questions were put according to the domain, namely the type, frequency and intensity of the activity performed, in the period covering the previous 12 months. Thus, a final score was obtained, and in accordance with Ueno,[25] the cutoff points for the level of physical activity were: low ≤ 9.11; moderate 9.12 to 16.17 and high ≥ 16.18.

Functional capacity of exercise

The functional capacity of exercise[26] in the elderly was evaluated by applying the 6MWT according to the protocol of the American Thoracic Society (ATS).[27] The results generated by the 6MWT were based on the reference values of Troosters et al.;[28] elderly individuals with the expected physical performance achieved ≥ 80% of the predicted values, while a low physical performance was attributed to those who achieved below 80%. It should be emphasized that 60 elderly patients did not undergo the 6MWT test as they were absent on the day it was applied.

Genetic analysis

To obtain the DNA, extraction was performed from peripheral blood leukocytes, acquired via venipuncture, in tubes containing EDTA (0.6%) and employing the Pure Link Genomic DNA extraction kit (Invitrogen, Carlsbad, USA), following to the letter the guidelines provided by the manufacturer. The extracted DNA was stored in a freezer at -80 °C awaiting the polymorphism analyses to be conducted. The quality and quantity of DNA was measured through an analysis of absorbance in a spectrophotometer (Thermo Scientific NanoDrop 2000)

at 260nm and 280nm. Then, the dilution of DNA was carried out in ultra-pure Milli-Q® water to obtain a final concentration of 30 ng/uL.

Polymerase chain reaction (PCR) and analysis of the polymorphism of the miRNA-146a gene

With the objective of analyzing the single nucleotide polymorphism (SNP) of the miRNA-146rs2910164, the technique of real time amplification of fragments of DNA by PCR was employed, using the TaqMan® system (Applied Biosystems, Foster City, USA). The reaction consisted of a final volume of 10 µL, namely: 5.25 µl of Taqman® Genotyping Master Mix (1x), 0.5µL of probe (1x) (Applied Biosystems, Foster City, USA), 3.25 µL of ultrapure water Milli-Q® and 1µL of DNA (30 ng/uL).[29]

The Thermocycler StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, USA) was used with a cycle of 60 °C for 30 seconds (initial denaturation), 95 °C for 10 minutes for initial denaturation, 50 cycles of 95 °C for 15 seconds (denaturation) and 60 °C for 90 seconds (pairing of initiators and extension) and a final extension cycle of 30 seconds at 60 °C.[29,30]

Statistics

The Statistical Package for the Social Sciences - SPSS (v.17, SPSS Inc., Chicago) was used to analyze the data obtained. The normality of the data was verified using the Shapiro-Wilk test. The absolute and relative frequencies for each variable were calculated. The Chi-square test (χ^2) was used to analyze the association between the dependent variable (CVD) and the independent variables: age, gender, race, BMI, systemic arterial hypertension (SAH), LDL, HDL, cholesterol, triglycerides, allele and genotype frequencies. For all the analyzed data, the level of significance was set at $p < 0.05$ with a confidence interval of 95%.

Results

Of the 506 seniors participating in the project, 164 did not attend every data collection. They were, therefore, excluded from the sample in order not to compromise the analyzed data. Of the 342 participating individuals, 39.8% were over the age of 70; 72.8% were women; 61.4% were caucasian. The majority of survey participants (64%) were hypertensive (Table 1).

Table 1 - Distribution of demographic, health and genetic characteristics in the studied population (n = 342)

Characteristics		n	%
Gender	Male	247	72.8
	Female	93	27.2
Age	60-64 years	86	25.1
	65-70 years	120	35.1
	>70 years	136	39.8
Race	White	210	61.4
	Brown	72	21.1
	Black	46	13.5
	Yellow	14	4.1
BMI	Underweight	6	1.8
	Normal	84	24.6
	Overweight	131	38.3
	Obese	120	35.1
LDL	Excellent/desirable	220	64.3
	Borderline	83	24.3
	High/Very High	37	9.9
HDL	Desirable	317	92.7
	Low	20	5.8
Cholesterol	Excellent/desirable	164	48.7
	Borderline	116	34.4
	High/Very High	57	16.9
Triglycerides	Desirable	198	58.8
	Borderline	75	22.3
	High/Very High	64	19.0
Cardiovascular disease	Present	218	63.7
	Absent	124	36.3
Hypertension	Hypertensive	132	35.9
	Non-hypertensive	74	64.1
Diabetes mellitus	Diabetic	109	31.9
	Non-diabetic	110	32.2
	Pre-diabetic	118	34.5
6MWT	Predicted (> 80%)	247	87.6
	Lower than the predicted (< 80%)	35	12.4
mBQ (level of PA)	Low	279	84.8
	Moderate	41	12.5
	High	9	2.7
Genotype	Homozygous CC	41	12.0
	Heterozygote CG	137	40.1
	Homozygote GG	164	48.0

Note: BMI = body mass index; LDL = low-density cholesterol; HDL = high-density cholesterol; PA = physical activity; 6MWT = 6-minute walk test; mBQ = Baecke Questionnaire modified for the elderly.

In relation to CVD, 63.7% of the elderly presented with the disease; 87.6% of those who were evaluated through 6MWT performed as predicted, while 84.8% achieved a low index of physical activity, as observed through the Baecke questionnaire. It was ascertained that 48% of the elderly were carriers of the genotype GG for the miRNA-146A gene (Table 1).

Meanwhile, of the elderly who did not present with the disease, 85% performed the 6MWT within the predicted parameters and 15% had a poorer result than predicted, not showing any relationship with the disease in question. No statistical difference was found between the presence of CVD and the following variables: age, gender, ethnicity, hypertension, diabetes mellitus, HDL, LDL, total cholesterol, triglycerides, 6MWT and genotype frequencies (Table 2). However, the BMI was associated with CVD ($\chi^2 = 14.278$; $p = 0.0003$); and 40.6% of elderly individuals with CVD were obese, while 31.5% of the normally developed elderly participants were free of CVD (Table 2).

Table 2 - Association between demographic and health characteristics, genotype frequencies, mBQ, TC6 and the presence or absence of cardiovascular disease (CVD)

Characteristics		with CVD n(%)	without CVD n(%)	Chi-square test	p-value
Age	60-64 years	55 (25.2)	31 (25.0)	2.953	0.228
	65-70 years	83 (38.1)	37 (29.8)		
	Over 70 years	80 (36.7)	56 (45.2)		
Gender	Female	166 (76.1)	83 (66.9)	3.387	-
	Male	52 (23.9)	41 (33.1)		
BMI	Underweight	1 (0.5)	5 (4.0)	14.278	0.003
	Normal	45 (20.7)	39 (31.5)		
	Overweight	83 (38.2)	48 (38.7)		
	Obese	88 (40.6)	32 (25.8)		
Triglycerides	Desirable	113 (52.8)	85 (69.1)	8.596	0.014
	Borderline	54 (25.2)	21 (17.1)		
	High	47 (22.0)	17 (13.8)		
Cholesterol	Desirable	105 (49.1)	59 (48.0)	3.985	0.136
	Borderline	79 (36.9)	37 (30.1)		
	High	30 (14.0)	27 (22.0)		
HDL	Desirable	198 (92.5)	119 (96.7)	2.497	0.114
	Low	16 (7.5)	4 (3.3)		
LDL	Desirable	142 (66.4)	78 (63.4)	0.450	0.798
	Borderline	52 (24.3)	31 (25.2)		
	High	20 (9.3)	14 (11.4)		
Genotype	Homozygous CC	23 (10.6)	18 (14.5)	1.211	0.546
	Heterozygote CG	88 (40.4)	49 (39.5)		
	Homozygote GG	107 (49.1)	57 (46.0)		
mBQ	Low level of PA	177 (84.3)	102 (85.7)	0.780	0.677
	Moderate level of PA	26 (12.4)	15 (12.6)		
	High level of PA	7 (3.3)	2 (1.7)		
6MWT	Predicted	156 (89.1)	91 (85.0)	1.025	0.311
	Lower than predicted	19 (10.9)	16 (15.0)		

Note: BMI = body mass index; HDL = high-density cholesterol; LDL = low-density cholesterol; mBQ = Baecke Questionnaire modified for the elderly; PA = physical activity; 6MWT = 6-minute walk test.

Discussion

Our study investigated, for the first time, the correlation between miRNA-146a and CVD in elderly people from a specific region of Brazil. Based on this principle, we sought to establish with greater authority the genetic characteristics of our population, since miscegenation is ubiquitous throughout the country.

We did not see a significant correlation between miRNA-146a and CVD, nor was there any significant correlation between CVD and 6MWT. However, we observed a significant correlation between BMI and CVD, as has been shown in other studies.[31,32] In this way, we can warn the population about the risks of this correlation and also reconfirm the data observed.

As for the genetic issue, miRNAs have fundamental characteristics for them to be considered good biomarkers; such as a high degree of sensitivity and specificity, essential to different physiological and pathological processes. Evidence shows that the miRNAs have the potential to be biomarkers of CVD, hypertension, heart failure, diabetes, stroke, among many other pathologies.[14]

However, this study showed that there was no association between CVD and the polymorphism in the miR-146a gene in an elderly South American population. These results corroborate those found by Li et al.,[33] who found no association between the rs2910164 polymorphism and age, gender, BP, cholesterol, 6MWT, DM in a population of 1,004 patients with coronary artery disease.

Ramkaran et al.,[34] who studied a young Iranian population, also found no association between CVD and genotype polymorphism miRNA-146a. The authors explained the findings were due to the number of individuals analyzed (n = 206) and the exclusion criteria, such as the BMI, arterial hypertension, diabetes mellitus and the effects of the drugs used.

However, the meta-analysis performed by Bao et al.[2] shows that rs2910164 is associated with a risk of CVD, and that the genotype GG and GG+GC or the G allele carry a lower risk for the development of the disease. However, the CC genotype does contribute to its development; but the authors also cite as limitations the reduced number of individuals, the scarcity of studies, limiting clinical-pathological characteristics, and the contribution of environmental factors which, in addition to genetic factors, are of great importance.

Although our study did not present significant results in relation to the C allele and CVD, Bao et al.,[2] Lung et al.[35] demonstrated that the C allele was classified as a predisposing disease allele, whereby its presence leads to lower levels of miRNA-146a due to the pre-processing of the microRNA which, thus altered, generates a reduction in the setting of target genes of miRNA 267a, causing the disease. Jazdzewski et al.,[36] moreover, affirms that the expression of pre-miRNA-146a of the C allele was 1.9 times lower than that of the G allele, and the amount of mature miRNA-146a was 1.8 times lower than the C allele compared to the G allele; thus, the decreased expression indicates that this single nucleotide difference modifies the amount of miRNA-146a produced from the C allele. Therefore, a polymorphism of the miRNA-146a that is involved in the inflammatory process may contribute to the development of CVD, as miRNA-146a is involved in the negative regulation of inflammation.[37]

In relation to functional capacity assessed through the six-minute walk test (6MWT) and CVD, no statistically significant relationship was found. However, Bautmans et al.[38] reported that, when analyzing healthy elderly subjects and elderly patients with risk factors for CVD, it was observed that healthy individuals produced a better physical performance than those with some change in their state of health; similarly, Pires et al.[39] reported that people with a BMI of less than 25 walk further than those with an index greater than 25; thus, the increased BMI may reflect on the performance in the Six-Minute Walk test.

The 6MWT is a very practical and low-cost option for evaluating aerobic capacity. In addition it encompasses an evaluation of daily, routine activities, since locomotion is the activity performed most frequently and with the greatest level of difficulty.[40]

One of the risk factors for reduced functional capacity and complications from various diseases is excess body fat since, the higher the percentage of body fat, the higher the body overload, causing limitations and joint and muscle stress in the elderly.[41]

In several studies, it was also ascertained that the practice of physical exercise at different intensities, which may or may not be controlled, generates many benefits for patients with CVD.[42] The benefits that physical exercise offers can be considered as the sum of efforts to modify cardiovascular risk factors, helping patients regain their functional independence in the community, leading to a more productive life.[43]

As regards BMI being associated with CVD, a statistically significant association was obtained; out of the 342 elderly subjects assessed, 73.4% were overweight or obese, of which 217 had CVD and, of these, 78.8% had a BMI above the standard of normality. The results are consistent with the Framingham Heart Study,[31] which reports that obesity is a significant risk factor for cardiovascular events, and Rabkin et al.,[32] who reported that the higher the BMI, the greater the possibility of cardiovascular risk factors.

It can be seen that the chronic accumulation of excess body fat leads to a variety of metabolic changes, increased risk for the development of CVD; as well as dyslipidemia, hypertension, glucose intolerance, inflammatory state, obstructive sleep apnea, and even structural adaptations.[44]

In another study, it was found that obesity is associated with several unfavorable physiological and hemodynamic changes. Obesity clearly increases the risk of CVD, but one should take into account those with low muscle mass, as they would enter into a category of predisposition to the disease.[45]

In a meta-analysis highlighting studies comparing lifestyle, CVD and other causes of death in elderly women, it was suggested that every 5 kg/m^2 increase in BMI might be associated with a 26% higher mortality risk among these women.[46]

Obesity, considered a risk factor for the development of CVD, implies that the lifestyle adopted by each individual may or may not contribute to the development of disease, and prevention methods such as adopting healthy habits, the practice of physical exercise and correct diet are of great importance.[47]

A limiting factor of this study is the sample number of 342 people which is small when it comes to analyzing polymorphisms, as a polymorphism is an allelic variation that appears in stable form in a population and must exhibit an incidence of at least 1%.[48] In addition, the sample may not have been sufficiently large to detect interactions among the variables. It is possible that, for more conclusive assertions about the genetic profile, one needs to consider a larger number of individuals and other variants of the miRNA-146a gene, which should be evaluated in combination through a study of the haplotype. Moreover, the quantification of the levels of transcripts of the miRNA-146a gene may confirm the hypothesis of the functional impact of this polymorphism.

Another issue to be considered is the population studied. Brazilian territory is composed of mixed populations, composed of people with ancestry from different regions of the world and bearers of distinct genetic characteristics.[49] Being a peculiarity of this population, the genetic study would result in a better interpretation and clarity about the health-disease process. Therefore, our study, being a pioneer in the area and in the evaluation of Brazilian elderly people, contributes to a better definition of genetic characteristics regarding the miRNA-146a polymorphism in relation to CVDs in elderly Brazilians.

Conclusion

Our results indicate that there is no association between CVD and the miRNA-146a polymorphism, nor functional capacity. However, the relationship between BMI and CVD is significant.

Authors' contribution

LRMP and LOL were responsible for the research and, along with DO, for the laboratory analysis. MC and RCPF analyzed the data, LRMP and PDOP wrote the manuscript, and PDOP and RCPF reviewed it. All authors approved the final version.

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